Seeding Cells into Calcium Phosphate Cement

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Introduction

We have sought to improve calcium phosphate cement by developing a method for seeding bone cells into the cement.

Calcium phosphate cement (CPC) is a dry white powder of calcium phosphate salts that when mixed with water will react to form microcrystalline hydroxyapatite (HA) in about 30 min[1]. The cement is used clinically as a bone graft for dental and craniofacial applications and its primary advantage is that it forms a workable paste that can be sculpted to fit the contours of a wound before it hardens into HA. Over time, the hardened CPC is converted to new bone (osseoconversion) by bone cells. We have sought to shorten the conversion time of the CPC by developing a method to seed live bone cells into the cement. The presence of bone cells in the cement would allow osseoconversion to begin throughout the implant as soon as the CPC is implanted. Initial experiments showed that the setting reaction of the CPC paste (but not hardened CPC) kills cells. Thus, we have tested the ability of alginate[2] to protect cells from the CPC paste during its 30 min setting reaction.

Materials and Methods*

Osteoblast-like MC3T3-E1 cells (Riken Cell Bank, Hirosaka, Japan) were cultured[3] in α modification of Eagle's minimum essential medium (Biowhittaker, Inc., Walkersville, MD) supplemented with 10 % (volume fraction) fetal bovine serum (Gibco, Rockville, MD) and kanamycin sulfate (Sigma, St. Louis, MO). Medium was changed twice weekly and cultures were passaged with 2.5 g/L trypsin (0.25 % mass fraction) containing 1 mmol/L EDTA (Gibco, Rockville, MD) once per week.

For experiments, cultures at 90 % confluency were trypsinized, counted in a haemocytometer, centrifuged, and resuspended in sterile-filtered 1.2 % sodium alginate (mass fraction) at 10^6 cells/mL [UP-LVG alginate (64 % guluronic acid) was obtained from Pronova (Oslo, Norway) and was dissolved in 0.9 % NaCl (mass fraction)]. Aliquots (500 μL) of the cell-alginate suspension were dropped from a 1 mL pipette into 8 mL of sterile $CaCl_2$ (100 mmol/L) in a 6-well plate and allowed to gel for 5 min. This procedure yielded spherical cell-alginate beads that were approximately 3.6 mm in diameter.

To test whether alginate can protect cells from the CPC setting reaction, a cell-alginate bead was packed into a sterile 6.4-mm-diameter mold containing CPC paste (0.25 g of CPC powder mixed with 62.5 μL of sterile water). CPC powder consists of equimolar amounts of ground $Ca_4(PO_4)_2O$ (72.9 % mass fraction) and $CaHPO_4$ (27.1 % mass fraction) and was pre-sterilized with UV-light for 24 h. CPC was a kind gift from S. Takagi and L. Chow (American Dental Association at NIST)

The mold containing the cell-alginate bead in CPC was placed in a 6-well plate with media (8 mL) in a cell incubator overnight. The next day, the cell-alginate bead was removed from the mold, washed with serum-free media, stained for 30 min in serum-free media containing live-dead stain (2 μmol/L calcein-AM and 2 μmol/L ethidium homodimer-1; Molecular Probes, Eugene, OR) and viewed by epifluorescence microscopy. Using the live-dead assay, live cells stain green and dead cells stain red. Control cell-alginate beads that were not exposed to CPC were also prepared and stained.

Results and Conclusions

A qualitative visual examination of Panels A and B in the Figure reveals that the density of live cells present in the 'Control' cell-alginate bead (Panel A) appears to be equivalent to the density of live cells in the 'CPC' cell-alginate bead (Panel B). Likewise, the density of dead cells present in the 'Control' bead (Panel C) appears to be equivalent to the density of dead cells in the 'CPC' bead (Panel D). Thus, beads that were packed into a mold with CPC paste such that they were in direct contact with CPC during its setting reaction contain the same amount of viable cells as control beads. These observations suggest that alginate protects cells from the CPC setting reaction. Thus, encapsulation of cells in alginate could be used as a mechanism to seed cells into calcium phosphate cement.

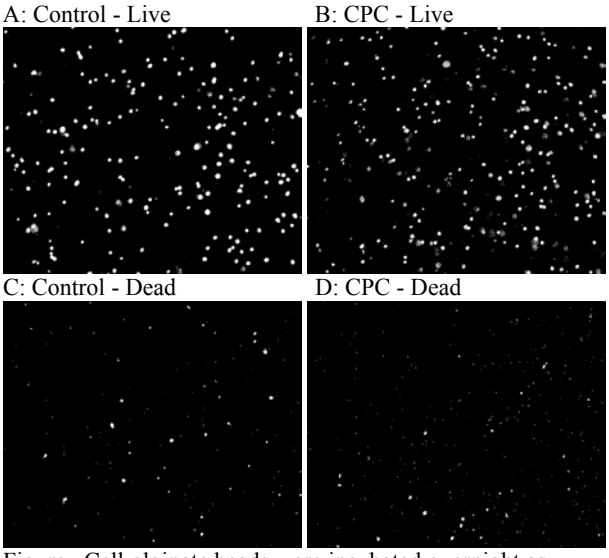

Figure. Cell-alginate beads were incubated overnight as controls (A & C) or seeded into CPC and incubated overnight (B & D). The next day, the beads were double-stained with calcein-AM (stains live cells green) and ethidium homodimer-1 (stains dead cells red) and observed using epifluorescence. Panels A & C are images of the same field in a 'Control' cell-alginate bead viewed with a green (A, live cells) or red (C, dead cells) filter. Panels B & D are images of the same field in a 'CPC' bead viewed with a green (B, live cells) or red (D, dead cells) filter. The panels are 517 μm by 427 μm.

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References

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